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Copper removal from water using a bio-rack system either unplanted or planted with *Phragmites australis*, *Juncus articulatus* and *Phalaris arundinacea*

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ABSTRACT

A bio-rack system was developed for treating Cu-contaminated freshwaters. Each pilot constructed wetland (CW, 110 dm^3) contained 15 perforated vertical pipes filled with a mixture of gravel (diorite; 80%) and perlite (20%) and assembled as a rack. The whole experimental device consisted of 12 CW planted either with *Phragmites australis*, *Phalaris arundinacea* or *Juncus articulatus*, and unplanted as control (in triplicates). All plants were sampled at a Cu-contaminated site. The CWs were filled with a mix of freshwater (30%) from the Jalle d'Eysines River (Bordeaux, France) and tap water (70%). Water was spiked with Cu (2.5 μM, 158.5 $\mu g\,L^{-1}$). Three CW batches were carried out, i.e. in early spring (March, S#1), beginning of the growing season (May, S#2), and peak growing season (June, S#3). The S#3 water was initially acidified to pH 6. For all batches, water was recirculated in the CW during 14 days. Physico-chemical parameters (pH, electrical conductivity, redox potential, BOD_5 and Cu^{2+} concentrations) were measured every three days. Water pH of both S#1 and #2 ranged between 7.8 and 8.5 for all treatments during the experiment. Initial and final total Cu concentrations were analysed for all CWs and batches. Relative Treatment Efficiency Index (RTEI) indicated the plant effect compared to the unplanted CW. Free Cu^{2+} removal was <10% for all S#1 treatments (RTEI ranged between 0 and −1) whereas it increased to 77% (RTEI = 0.1) in S#2 for *P. arundinacea*. In acidic conditions (S#3), Cu^{2+} removal was 99% for all treatments (RTEI = 0). For S#1 and S#2, highest total Cu removal occurred in CW planted with *P. arundinacea* (respectively 52% and 68%, RTEI = 0.1 and 0.2). For S#3, total Cu removal peaked up to 90% in the unplanted CW. The RTEI values suggested no beneficial effect of macrophytes on Cu removal at short term. Conversely, the CW planted with *J. articulatus* generally displayed a lower efficiency. The lowest value for total Cu concentration in water after the 14-day period was 13 $\mu g\,L^{-1}$ in S#3 unplanted and planted with *P. arundinacea*. The role of the biofilm as a key-player of Cu removal in such bio-racks is discussed.

1. Introduction

1.1. Constructed wetlands (CW)

Water quality issues are a major challenge faced by mankind in the 21st Century (Corcoran et al., 2010). Treatment of municipal wastewater streams aims at eliminating nutrients, pathogenic microbes, persistent organic pollutants, xenobiotics derived from the pharmaceutical industry and trace elements (TE) from wastewater streams (Schwartzenbach et al., 2010). In industrialized countries, connection to municipal wastewater treatment plants ranges from 50% to 95%, whereas more than 80% of the municipal wastewaters in low-income countries are discharged without any treatment, polluting rivers, lakes, and coastal sea areas (UNESCO, 2009). As a consequence, pollutants accumulate in aquatic ecosystems in surface waters, groundwater, substrates and plants (Aksoy et al., 2005; Demirezen et al., 2007; Lizama et al., 2011, 2012). In Bordeaux area (France), Cu is one of the major contaminant since soluble formulations of Cu-sulphate and chromated Cu-arsenate (CCA)-type C are used as treatment agents against insects and

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fungal attacks in vineyards and timber harvesting industries (Bes et al., 2013).

Constructed wetlands (CW) planted with macrophytes are an emerging phytotechnology frequently used as an efficient and cost-effective alternative for treating wastewater streams due to its low energy requirements, its convenient operation, and weak maintenance (Marchand et al., 2010; Vymazal, 2010; Hsu et al., 2011; Kuschk et al., 2012; Haarstad et al., 2012; Adams et al., 2012). Five mechanisms affect TE removal in natural and constructed wetlands (Sheoran and Sheoran, 2006; Lesage et al., 2007; Marchand et al., 2010): (1) sorption to fine textured sediments and organic matter, (2) (co)precipitation as insoluble salts, mainly sulphides in reducing conditions and Fe/Mn/Al (oxy)hydroxides in oxidative conditions, (3) carbonate (co)precipitation, (4) absorption and induced changes in biogeochemical cycles by plants and associated micro-organisms (fungi and bacteria of the rhizosphere as well as endophytes), and (5) deposition of suspended solids due to low flow rates. All these reactions lead to metal accumulation in the wetland substrate. The CW efficiency depends on inlet metal concentrations, hydraulic loading, pH, redox conditions and the presence/absence of the consortium plant/bacteria (Kadlec and Wallace, 2009).

1.2. Controversial role of macrophytes

Macrophytes are key-players in CW by driving TE uptake and storage in roots and rhizomes (Caldelas et al., 2012), as well as by TE sorption onto the root plaque (McCabe et al., 2001) and into the substrate by releasing organic metal ligands (Ryan et al., 2001). They also contribute to maintain oxidative conditions in the rhizosphere (Armstrong, 1978; Stottmeister et al., 2003; Cheng et al., 2009) and supply plant-derived organic matter over time which continuously provides sites for metal sorption, as well as carbon sources for bacterial metabolism, thus promoting long-term functioning (Jacob and Otte, 2004; Huang et al., 2012; Leto et al., 2013). Although macrophytes are widely used within CW (Marchand et al., 2010), their role and the effect of different plant species on the CW has been controversial, mainly in terms of TE removal (Lee and Scholz, 2007; Brisson and Chazarenc, 2009; Marchand et al., 2010). Moreover, TE uptake by macrophytes largely depends on the vegetative periodic variations (Bragato et al., 2006; Baldantoni et al., 2009; Wu et al., 2013). In this study, the role of the three macrophytes *Phragmites australis* (Cav.) Trin. ex Steud, *Juncus articulatus* L. and *Phalaris arundinacea* L. in Cu removal from water was assessed across the vegetative season in planted bio-racks filled with substrate.

1.3. Bio-racks

Common classification divides wetlands according to their hydrology: (1) Surface Flow Wetlands (SF), (2) Horizontal Sub-Surface Flow wetlands (HSSF), (3) Vertical Sub-Surface Flow wetlands (VF), and (4) Hybrid Systems (HS) (Arias and Brix, 2003). A new CW design was proposed by Valipour et al. (2009), the so called bio-rack system. Unique feature of this system was the presence of numerous vertical pipes, free of sediment but planted with *P. australis*, assembled as a (bio)rack, which is meant for holding vegetation and support matrix for bacterial growth. Here, bio-racks were filled with substrate to take advantage of both systems, i.e. in the conventional CW the substrate provides adsorption sites for TE, while bio-racks provide maintenance facilities by allowing a rack turnover over time. Such turnover may avoid clogging due to OM accumulation, biofilm development and saturation of sorption sites.

2. Materials and methods

2.1. Pilot plant setup

A pilot plant was built in a greenhouse located at the National Institute for Research in Agronomy (INRA, Villenave d'Ornon, 44°46′50″ N 0°33′57″ W, France) and started its operation in January 2011. Experiments were carried out in twelve independent polyethylene tanks: 32 cm × 56 cm surface opening, 36 cm depth, and containing a 60 dm^3 volume at 34 cm operational water depth (Fig. 1). Each tank was connected to a storage tank (total volume: 60 dm^3, 50 dm^3 at operational water depth). For convenience, we will refer to the polyethylene tanks as "unit A", storage tank as "unit B" and the whole as a pilot constructed wetland (CW) throughout the paper. In December 2010, each unit A was filled with 15 vertical PVC pipes (diameter: 10 cm, depth (H): 35 cm, volume (V): 2.75 dm^3) assembled as a rack termed as "bio-rack". All vertical pipes were perforated every five cm in height and width (hole diameter: 5–10 mm) to enable liquid transport and root development out of the pipe, and – contrary to Valipour et al. (2009) – filled with a homogeneous mix of gravels (diorite, 1–5 mm, 3.9 kg) and perlite (0.035 kg) (respectively 80% (v/v) and 20% (v/v)). Porosity of the substrate made of gravels and perlite was 36%, thus the water volume into each pipe was 1 dm^3. Each bio-rack occupied 41 dm^3 of the unit A and contained 15 dm^3 of water and 26 dm^3 of substrate. The 19 dm^3 remaining in each unit A were occupied by free water. Total water volume in each unit A was thus 34 dm^3. Water volume in each unit B was 50 dm^3. The units A were connected to the units B using lift pumps (Vc400ech, 7500 $dm^3\ h^{-1}$, Leroy Merlin, China) (Fig. 1). Total water volume in each CW was 84 dm^3.

2.1.1. Plants

Plants were collected in 2010, at the beginning of the growing season (April–May), at the La Cornubia site (44°54′26″ N; 0°32′46″ W, Bordeaux, France), a former chemical plant producing Cu sulfate and Cu-based fungicides, dating back to a century and closed in 2004. *P. arundinacea* L. and *J. articulatus* L. were sampled in an abandoned constructed pond colonized by macrophytes, connected to a pipe collecting storm water and effluents from the plant. Total soil Cu and soil pH at this sampling sub-site are respectively 205 mg kg^{-1} DW and 5.7. *P. australis* were sampled on the riversides of a small creek that borders this chemical plant, contaminated by effluents, surface runoff, stormwater, and dust fallout. *P. australis* and *J. articulatus* are rhizomatous geophytes, having shoots borne from buds in the soil and resting buds lying beneath the soil surface as rhizomes. *P. arundinacea* is a hemicryptophyte; it exhibits buds either at or near the soil surface (Raunkiær, 1934). Plants of each population were separately kept in buckets and immediately transported to a greenhouse (INRA – Centre Bordeaux Aquitaine, Villenave d'Ornon, S1). The next day, rhizomes and/or stems bearing buds were cut into small pieces (10–20 cm). They were then individually grown during nine months (May 2010-January 2011) in plastic pots placed in polyethylene vats (volume: 60 cm × 40 cm × 15 cm) containing perlite imbibed with tap water and a quarter Hoagland nutrient solution (HNS, Hoagland and Arnon, 1950): KNO_3 (1.62 mM), $Ca(NO_3)_2$ (0.69 mM), $NH_4H_2PO_4$ (0.25 mM), $MgSO_4$ (0.5 mM), H_3BO_3 (11.5 μM), $MnCl_2$ (2.29 μM), $CuSO_4{\cdot}5H_2O$ (0.08 μM), $(NH_4)_6Mo_7O_{24}$ (0.13 μM), $ZnSO_4$ (0.19 μM), and $Fe^{(II)}SO_4$ (48.6 μM). Water volume was maintained constant by tap water addition and monthly changed to avoid anoxia, with addition of 1 dm^3 of a quarter HNS to avoid nutrient depletion in the growing medium. Water was changed every two months during winter. In January 2011, rhizomes and stems bearing buds were cut again into small pieces (5–10 cm). Three unplanted CW were used as controls. Other CWs were planted (in

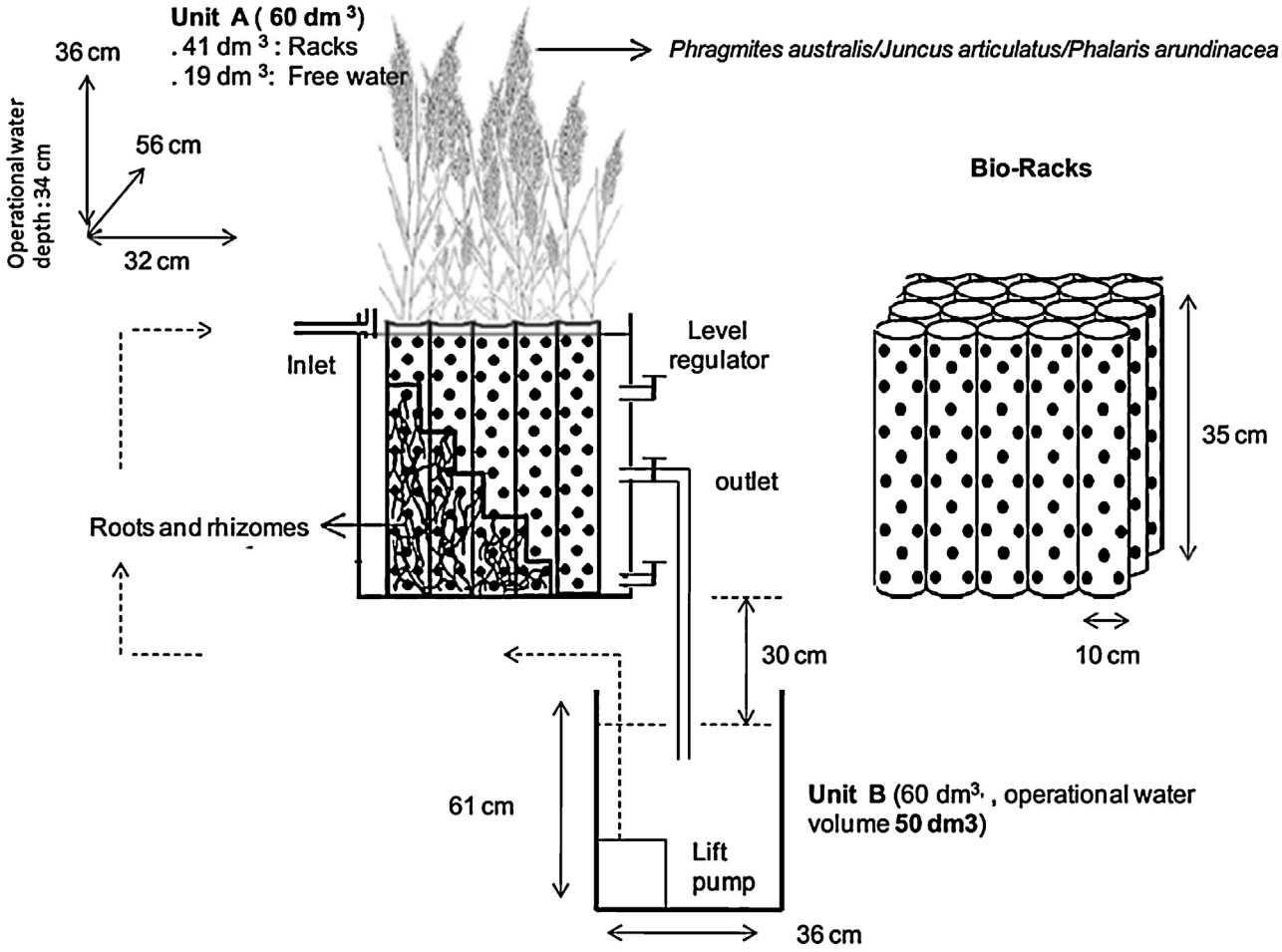

Fig. 1. Scheme of the constructed bio-rack wetland.

Adapted from Valipour et al. (2009).

triplicates), respectively with *J. articulatus*, *P. arundinacea* and *P. australis*. Two plant pieces were transplanted 5 cm beneath the substrate surface of each vertical pipe. Units A were then filled with tap water (34 dm^3). Between January 2011 and March 2012, the water was changed every six weeks with addition of a quarter HNS (2 dm^3). Sodium and Fe salt of EDDHA (Sequestrene, Syngenta) was added in units A once at mid-June (10 mg dm^{-3}, 23 μM) to promote plant Fe nutrition. Water level was maintained in the system by tap water addition. In December 2011, the dried aboveground biomass was harvested at 20 cm above the bio-rack surface. These CWs were carried out from January 2011 to March 2012 for allowing plants to produce sufficient belowground biomass in such greenhouse conditions.

2.1.2. Sampling and analysis

In February 2012 (weeks 8 and 9), CW were filled with tap water and a quarter HNS (2 dm^3), and spiked with 1 μM Cu (63.5 μg Cu dm^3 by addition of 21 mg $CuSO_4{\cdot}5H_2O$ in 84 dm^3) for allowing progressive macrophyte adaptation to Cu contamination and Cu^{2+} sorption on binding sites. Then, CWs were filled again with tap water and 2 dm^3 of a quarter HNS during weeks 10–12. The day prior the experiment, CW were emptied and then filled with 70% of tap water and 30% of freshwater from the Jalle d'Eysine River. This urban river is located in southwest France (44°53′36″ N; 00°40′40″ O), North of Bordeaux, and a tributary of the Garonne River. From its headwaters to its confluence with the Garonne River, it is 32 km long. Water depth typically varies from 0.8 to 2.5 m annually, and average water flow is 3 m^3 s^{-1}. It receives TE-contaminated runoff from industrial, agricultural and residential areas and effluents from two major municipal wastewater treatment plants (WTP) that serve more than 100,000 inhabitants in the Bordeaux suburbs. Treated effluents can account for up to 33% of the river flow (Labadie and Budzinski, 2005). Electrical conductivity (EC) in this freshwater ranges from 0.48 to 0.94 Ms cm^{-1}, pH from 6.8 to 7.6, and TE concentrations are below the detection limit (Cu < 8, Zn < 7, Cd < 0.1, Cr < 0.3, and Pb < 0.8 μg dm^3) (personal data). Addition of freshwater in the CW mainly provided nutrients and microorganisms and avoided to supply HNS (**S4**). Conditions in the CW were thus closer to contaminated freshwaters. Experiments were carried out during 14 days, and water was daily recirculated 10 h day^{-1}, from 8:30 am to 6:30 pm. Inlet and outlet water flows were 5.3 dm^{-3} min^{-1}. For the 1st experiment (S#1, weeks 13 and 14), on day 1 (23/03/2012), CW were spiked with 2.5 μM Cu (158.5 μg Cu dm^{-3} by addition of 52.4 mg $CuSO_4{\cdot}5H_2O$ in 84 dm^3). Immediately (T_0), four water samples (100 cm^3) were collected in all CW (i.e., two in unit A and two in unit B), resulting in a total of 48 samples (12 per modality). Such water sampling was repeated on days 3, 6, 10, and 14. The O_2 concentration, EC and redox potential were measured with a WTW Multiline P4 metre (Germany) in 24 samples, i.e. six for each modality, at T_0 as well as pH (Hanna instruments, pH 210, combined electrode Ag/AgCl, USA) and Cu^{2+} concentrations (Fischer Bioblock Cupric Ion Electrode, USA) after addition of 2 cm^3 of $NaNO_3$ (5 M).

Table 1
Physico-chemical parameters, i.e. pH, Eh (mV), EC ($\mu S\,cm^{-1}$) and BOD_5 ($mg\,dm^3$) in CW at day 0 and day 14 ($n = 6$).

	Control	*J. articulatus*	*P. arundinacea*	*P. australis*	Control	*J. articulatus*	*P. arundinacea*	*P. australis*
	pH				**Eh (mV)**			
S#1 (Cu =2.5 μM)								
Day 0	8.0 ± 0.1	8.0 ± 0.1	7.8 ± 0.1	7.9 ± 0.1	218.6 ± 1.9	220.2 ± 1.2	224.6 ± 2.6	222.8 ± 1.5
Day 14	8.4 ± 0.1	8.3 ± 0.1	8.3 ± 0.1	8.3 ± 0.1	172.8 ± 1.5	174.8 ± 1.9	176.3 ± 2.6	182.8 ± 3.6
S#2 (Cu = 2.5 μM)								
Day 0	7.9 ± 0.1	7.9 ± 0.2	7.9 ± 0.1	7.9 ± 0.1	232.8 ± 3.6	232.3 ± 3.4	237.2 ± 5.3	235.3 ± 3.9
Day 14	8.4 ± 0.1	8.4 ± 0.1	8.4 ± 0.1	8.4 ± 0.1	210.5 ± 2.2	213.6 ± 1.9	206.2 ± 2	210.3 ± 1.5
S#3 (Cu = 2.5 μM)								
Day 0	6.1 ± 0.2	6.1 ± 0.1	6.2 ± 0.2	6.1 ± 0.1	215.1 ± 12.6	218.5 ± 5.8	229.0 ± 6.1	235.2 ± 24.5
Day 14	7.8 ± 0.1	7.7 ± 0.1	8.1 ± 0.1	7.9 ± 0.1	218.6 ± 6.2	224.3 ± 2.9	218.8 ± 3.9	212.8 ± 2.8
	EC ($\mu S\,cm^{-1}$)				**BOD_5 ($mg\,O_2\,dm^{-3}$)**			
S#1 (Cu =2.5 μM)								
Day 0	441 ± 1.5	448 ± 2.8	447.2 ± 4.5	441.6 ± 1.7	1.2 ± 0.1	1.4 ± 0.18	1.4 ± 0.2	1.4 ± 0.3
Day 14	487.6 ± 5.7	521.5 ± 14.3	487.3 ± 7.4	501.8 ± 5	<1	<1	<1	<1
S#2 (Cu = 2.5 μM)								
Day 0	425.3 ± 3.9	439 ± 20.7	429.5 ± 3.4	430 ± 6.4	<1	<1	<1	<1
Day 14	451.3 ± 2.7	485.5 ± 9.8	449.5 ± 3.9	471 ± 20.1	<1	<1	<1	<1
S#3 (Cu = 2.5 μM)								
Day 0	706.7 ± 67.8	562.8 ± 8.1	554.8 ± 16.7	574.6 ± 6.9	2.01 ± 0.1	2.1 ± 0.3	2.5 ± 0.2	2.23 ± 0.4
Day 14	850.2 ± 96.5	681 ± 14.5	653.2 ± 10.7	642.8 ± 6.4	<1	<1	<1	<1

All measurements were performed on the day of water sampling. The remaining 24 samples, i.e. six for each modality, were kept for 5 days at 20 °C, in dark conditions, and then O_2 concentration ($mg\,dm^{-3}$) was measured at T_5. The 5-day biological oxygen demand (BOD_5) was calculated as $BOD_5 = [O_2]\,T_0 - [O_2]\,T_5$. Total Cu concentration was analysed in these water samples at day 0 and day 14 by ICP-AES (Varian liberty 200) and ICP-MS (Thermo X series 200) at the INRA USRAVE laboratory, Villenave d'Ornon, France. At day 15, CW were emptied, and rinsed two times with tap water. Units B and pumps were rinsed a third time, wiped with absorbing paper and put to air dry during two days. Then, CWs were filled again with tap water and 2 dm^3 of a quarter HNS. This experiment was duplicated during weeks 19 and 20 (S#2), when the growing season peaked under the greenhouse conditions. The third experiment (S#3) was carried out during weeks 26 and 27, but the water (i.e. 1/3 freshwater + 2/3 tap water) was acidified with 1 M HNO_3 the day prior the addition of 2.5 μM Cu. Nitric acid was progressively added into all CW to reach a pH of 5.5. After one reaction day, pH at T_0 rose up to 6.1 (Table 1).

2.1.3. Statistical analysis

The Cu removal rate (expressed in %) was computed as $100 - ([Cu]_{14}/[Cu]_0) \times 100$, where $[Cu]_0$ was the initial Cu concentration in the CW (2.5 μM at D_0) and $[Cu]_{14}$ the final Cu concentration measured at D_{14}. The Relative Treatment Efficiency Index (RTEI) was used to assess the macrophyte effect on the Cu removal rate in CW planted with *P. arundinacea*, *J. articulatus* and *P. australis* compared to the unplanted CW (Marchand et al., 2010).

$$\mathrm{RTEI} = \frac{T - C}{T + C}$$

where T (%) is the Cu removal rate in a planted CW and C (%) the Cu removal rate in the unplanted CW (Table 2).

The effect of macrophytes planted in bio-racks during the 14-day period on Cu^{2+} (Fig. 2) and total Cu (Fig. 3) removal was tested using one-way analysis of variance (ANOVA). Normality and homoscedasticity of residuals were met for all tests. Post hoc Tukey HSD tests were performed to assess multi-comparisons of means. Differences were considered statistically significant at $p < 0.05$. All analyses were carried out using R software (version 2.14.1 R foundation for Statistical Computing, Vienna, Austria).

3. Results and discussion

Conventional root zone system in CW using multilayered bed from stone to mud may result in clogging of interstices (Valipour et al., 2009). The roots of *P. australis* can poorly grow in such bed conditions due to perforation difficulties (Davison et al., 2005). A modified root zone system with a distinguished surface providing sites for the growth of microbial bio-films can facilitate either the sorption or degradation of many pollutants through (bio)chemical reactions (Valipour et al., 2009). In our bio-racks (Fig. 1), vertical pipes filled with a mix of gravels and perlite provided potential sorption sites for Cu onto the substrate, free roots (including their Fe/Mn plaques) and rhizomes growing through the holes, microorganisms and their biofilms. Moreover, columns can be replaced periodically to offer new sorption sites and indeed rejuvenate the CW. This system allowed removing up to 99% of the Cu^{2+} and 90% of the total Cu from Cu-contaminated waters under acidic conditions (Figs. 2 and 3, Table 2).

3.1. Physico-chemical parameters and Cu removal in bio-racks during a 14-day period in alkaline conditions

The S#1 and S#2 experiments were carried out at pH 8, in early spring and during the growing season. At day 0, for both experiments, pH was in the range [7.8 ± 0.07 to 8 ± 0.07] in all CWs (Table 1). Then, it slightly increased at day 3 to [8.2 ± 0.2 to 8.4 ± 0.07] and remained stable until the exposure end. At the end of both experiments (day 14), pH varied between [8.3 ± 0.06 to 8.4 ± 0.08]. Therefore, pH conditions were similar for all CW during S#1 and S#2. Redox potential ranged respectively between [172.8 ± 1.5 to 218.6 ± 1.9 mV] during the S#1 and [210.5 ± 2.2 to 233 ± 2.7 mV] during the S#2. Redox conditions were oxidative in all CW treatments and slightly increased as a function of time (Table 1). Electrical conductivity also slightly increased; it respectively ranged from 441 ± 1.5 to 487.6 ± 5.7 $\mu S\,cm^{-1}$ for the S#1 and between 425.3 ± 3.9 and 451.3 ± 2.7 $\mu S\,cm^{-1}$ for the S#2. Total Cu was respectively 0.7 μM (44 ± 9 $\mu g\,dm^{-3}$) and 0.8 μM (48 ± 7 $\mu g\,dm^{-3}$) in CW at the beginning of both first experiments (Fig. 3). In such alkaline conditions, free Cu is under the form Cu^{2+} but also $CuCO_3{}^0$ (Wang et al., 2012). The major part (70%) of the 2.5 μM Cu added in S#1 and S#2 was immediately sorbed

Table 2
Rates of Cu^{2+} and total Cu removals (%), and RTEI of bio-racks during after a 14-day period (from 23/03 to 05/04: S#1, 03/05 to 17/05: S#2, and 22/06 to 06/07: S#3 in 2012).

Treatments	Day	S#1		S#2		S#3	
		%Removal	RTEI	%Removal	RTEI	%Removal	RTEI
Cu^{2+}							
Control	14	10	–	62	–	99	–
Juncus articulatus	14	<0	−1	47	−0.1	99	0
Phragmites australis	14	10	0	72	0.1	99	0
Phalaris arundinacea	14	2	−0.7	77	0.1	99	0
Total Cu							
Control	14	40	–	49	–	90	–
Juncus articulatus	14	11	−0.5	11	−0.6	82	−0.1
Phragmites australis	14	7	−0.7	35	−0.2	85	0
Phalaris arundinacea	14	52	0.1	68	0.2	87	0

as insoluble form onto the perlite, Fe oxides of diorite, the microbial biofilms, macrophyte roots and PVC layers. Consequently, both Cu removal (in %) and Cu^{2+} removal (in %) were calculated respectively based on the total Cu and the free Cu^{2+} in water at day 0. In S#1, total Cu removal at day 14 reached 40% in the unplanted CW, 11% in the CW planted with *J. articulatus* (RTEI = −0.5), 7% in the CW planted with *P. australis* (RTEI = −0.7) and 52% in CW planted with *P. arundinacea* (RTEI = 0.1) (Table 2). Similar trend occurred for the S#2, the lowest and the highest total Cu removals being respectively obtained in CW planted with *J. articulatus* (11%, RTEI = −0.6) and *P. arundinacea* (68%, RTEI = 0.2). Total Cu removal in the CW planted with *P. australis* increased during the S#2 (35%, RTEI = −0.2). Highest total Cu concentrations in water at day 14 were found for both S#1 and S#2 in CW planted with *J. articulatus* (respectively, 0.8 μM, 48.3 ± 9 μg Cu dm^{-3} and 0.75 μM, 47.5 ± 5 μg Cu dm^{-3}) while the lowest ones occurred in CW planted with *P. arundinacea* (respectively 0.3 μM, 19.7 ± 18 μg Cu dm^{-3} and 0.2 μM, 15.5 ± 7 μg Cu dm^{-3}) (Fig. 3). Similar patterns were quantified for free Cu^{2+} removal. At the beginning of S#1 and S#2, free Cu^{2+} concentration in water was 0.025 μM (1.6 ± 0.13 μg dm^{-3}). At day 14 of S#1, water Cu^{2+} concentrations did not differ across treatments (from 0.02 μM, 1.23 ± 0.11 μg Cu dm^{-3} in control to 0.03 μM, 1.81 ± 0.17 μg Cu dm^{-3} in CW planted with *J. articulatus*). These values were similar to the initial concentrations (day 0) (Fig. 2). Thus, Cu^{2+} removal ranged from 0% in CW planted with *J. articulatus* (RTEI = −1) to 10% in both unplanted CW and CW planted with *P. australis* (RTEI = 0) (Table 2). For S#2, Cu^{2+} removal was more efficient in all treatments. At day 14, free Cu^{2+} removal reached 77% in CW planted with *P. arundinacea* (RTEI = 0.1, final concentration: 0.01 μM, 0.6 ± 0.05 μg Cu^{2+} dm^{-3}), while its lowest value was found for CW planted with *J. articulatus* (17%, RTEI = −0.1, final concentration: 0.014 μM, 0.9 ± 0.1 μg Cu^{2+} dm^{-3}) (Table 2).

The pH influences the efficiency of metal removal in wetlands (Sheoran and Sheoran, 2006; Marchand et al., 2010). When pH is in the [8–9] range, Cu coprecipitation with sulphides and/or hydrogen sulphides may occur under reducing conditions in the presence of OM (Brookins, 1988; Sheoran and Sheoran, 2006). Under oxidative conditions, the overall mean surface charge of

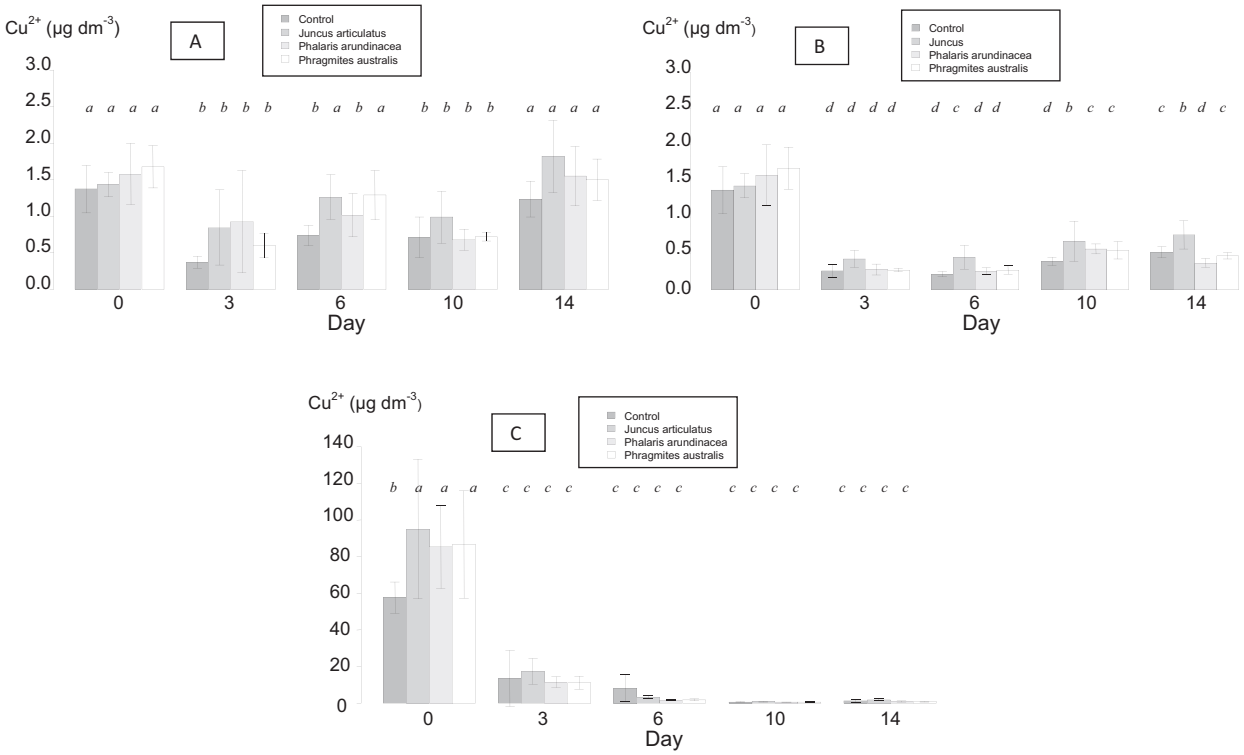

Fig. 2. Water Cu^{2+} concentrations (μg dm^{-3}) in CW during a 14-day period. (A) S#1, (B) S#2, and (C) S#3 ($n = 6$). The different letters stand for statistical significance at the 0.05 level with the Tukey HSD test.

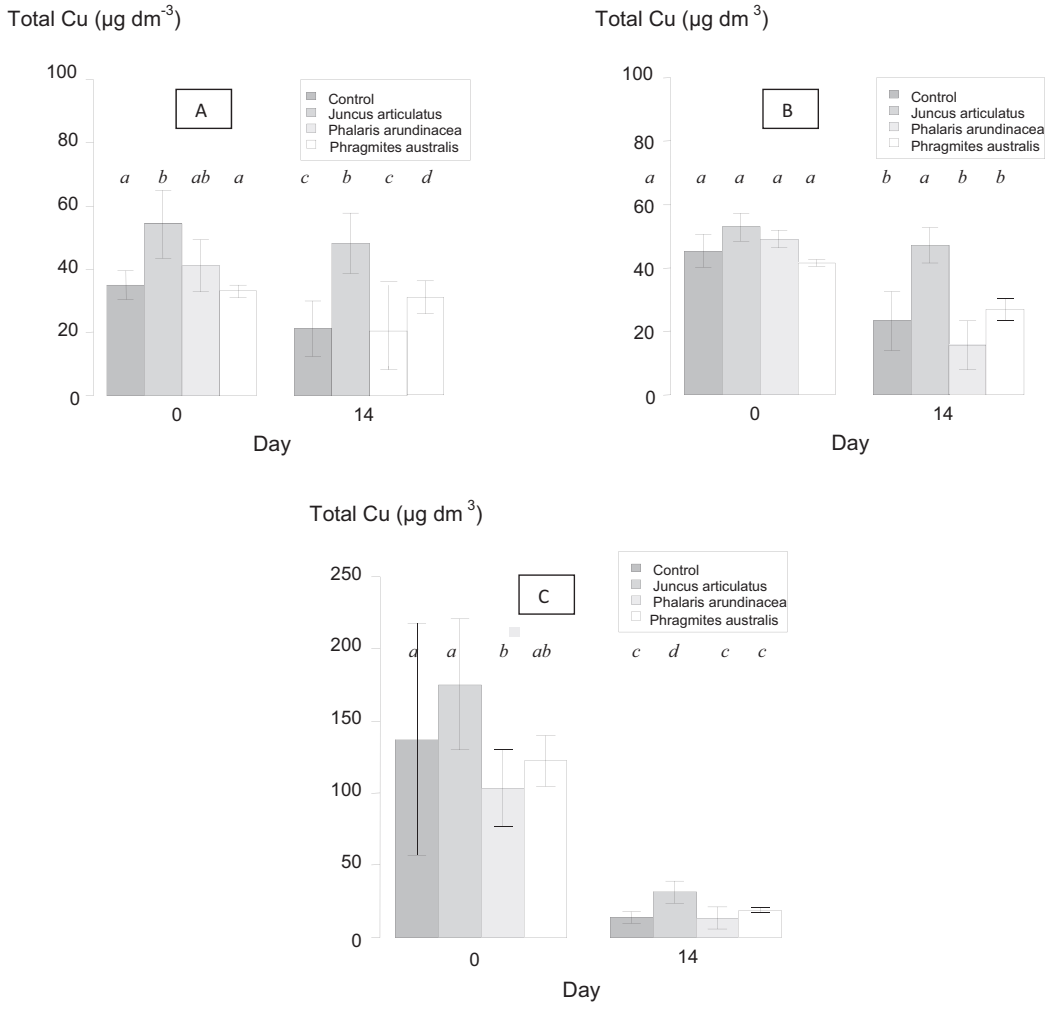

Fig. 3. Total Cu concentrations (mg dm^{-3}) in CW water at day 0 and day 14 ($n = 4$): (A) S#1, (B) S#2 and (C) S#3. The different letters stand for statistical significance at the 0.05 level with the Tukey HSD test.

ferric (oxyhydr)oxides changes from a positive to a negative value as pH increases. Thus Cu-coprecipitation with (oxyhydr)oxides takes place in CW at pH > 7 (Brookins, 1988; Sheoran and Sheoran, 2006; Marchand et al., 2010). Here, water pH values of S#1 and S#2 were 8, and redox conditions were oxidative due to water recirculation. Therefore Cu-coprecipitation with sulphides could not explain Cu removal, but it may have occurred with (oxyhydr)oxides.

Copper in our CW may also sorb onto Fe oxides from diorite and react with silicates derived from the perlite to form hydrated Cu silicates ($CuSiO_3 \cdot nH_2O$). Perlite was used for removing free Cu^{2+} ions from aqueous solutions (Alkan and Dogan, 2001; Sari et al., 2007). The amount of Cu^{2+} adsorbed rose as pH increased, whereas it diminished as the ionic strength, temperature and acid activation increased. Sari et al. (2007) reported a monolayer adsorption capacity of 8.62 mg Cu^{2+} g^{-1} on perlite. Here, the perlite in each bio-rack amounted to 534 g and thus 4 603 mg Cu^{2+} could adsorb on it in slightly alkaline conditions. All the Cu^{2+} added in S#1 and S#2 was not removed at day 14, and the bio-rack design might be involved. Diffusion through the substrate and contact with perlite may be limited by the PVC pipes. However, such limitation occurred also in S#3, whereas Cu removal increased, and can be likely ruled out. Other inorganic compounds may form such as $Cu_3(PO_4)_2$ since phosphates were present in the mixture of freshwater and tap water and in the HNS.

P. australis and *P. arundinacea* produce a large belowground biomass (Adams and Galatowitsch, 2005; Asaeda et al., 2006). Hence, they may take up in roots a significant Cu amount (Bonanno and Lo Giudice, 2010) and provide binding sites for Cu sorption onto the Fe/Mn plaque (Mantovi et al., 2003; Otte et al., 2004; Kissoon et al., 2010). Copper could also be immobilized under metallic nanoparticles in and near roots with assistance of endomycorrhizal fungi (Manceau et al., 2008). Additionally, graminaceous macrophytes such as *P. arundinacea* and *P. australis* excrete phytosiderophores which may complex free Cu^{2+} (Tsednee et al., 2012). However, for S#2 the removal rates of total Cu and free Cu^{2+} were similar in the unplanted CW and CW planted with *P. australis* and *P. arundinacea* (Table 2). Moreover, on the whole 14-day period, Cu^{2+} concentration was low (<10%) compared to total Cu concentration in all treatments. Consequently, Cu in recirculated water would mainly occur as CuO, Cu_2O and Cu bound with dissolved organic matter (DOM). This adds to conflicting data regarding plant influence on metal removal from water in studies comparing planted and unplanted CW (Lee and Scholz, 2007; Marchand et al., 2010). Alternatively, similar rates of Cu removal in the unplanted CW and CW planted with *P. australis* and *P. arundinacea* in S#2 may be due to the microbial biofilm established in the bio-racks before the experiments with Cu (Huang et al., 2000; Toner et al., 2005; Tanner and Headley, 2011; Valipour et al., 2009). Soil microbes are the

most important components of CWs (Huang et al., 2012). Microbial biofilms can trap Cu oxides such as Cu_2O and CuO (Paradies et al., 1996). Several months prior to the experiments, unplanted and planted bio-racks were supplied with a quarter HNS for allowing the macrophyte growth. Microbial biofilm may better develop in the unplanted CW without root competition for nutrients. Conversely, macrophyte roots can provide organic compounds such as polysaccharides to promote bacteria growth (Marchand et al., 2010). Huang et al. (2012) reported higher total nitrogen (Tn-N) removal number of soil microbes and activities of soil enzymes in

Table 3
Removal rates of Cu (%) in constructed wetlands.

Authors	Type of wetland	Plant species	Length × Width × depth (m)	Retention time (days)	Hydraulic loading rate (m^{-3} day^{-1})	Cu inlet ($\mu g\,dm^{-3}$)	Cu outlet ($\mu g\,dm^{-3}$)	Removal rate (%)
Crites et al. (1997)	SF, HSSF	*Schoenoplectus acutus*	10 cells, Total surface 384 × 15 × (0.15–0.6)	3–13	3785	7	3	55
Mantovi et al. (2003)	HSSF	*Phragmites australis*	2 cells in parallel, each of 12 × 6 × 1	10	6.3	81	17	79
Kamal et al. (2004)	SF (batch)	*Menta aquatica*	1 cell, 1.2 × 0.6 × 0.8	–	–	5560	3480	30.9
	SF (batch)	*Ludwigia palustris*[a]	1 cell, 1.2 × 0.6 × 0.8	–	–	5560	3060	44.9
	SF (batch)	*Myriophyllum aquaticum*[a]	1 cell, 1.2 × 0.6 × 0.8	–	–	5560	3190	42.5
Samecka-Cymerman et al. (2004)	SF	*Phragmites australis*	1 cell, 1200 m^2	–	–	W 7.9; S 6.2	W 4.5; S 2.7	W 43; S 56
	HSSF	*Salix viminalis*	1 cell, 1500 m^2	–	–	W 3.7; S 4.5	W 3.1; S 2.9	W 16; S 36
	HSSF	*Populus canadensis*	1 cell, 8000 m^2	–	–	W 4.1; S 7.3	W 2.1; S 1.9	W 49; S 60
Mishra and Tripathi (2009)	SF (batch)	*Eicchornia crassipes*[a]	1 cell, 10 dm^3	15	–	1000–5000	-	86–95
	SF (batch)	*Spirodela polyrrhiza*[a]	1 cell, 10 dm^3	15	–	1000–5000	-	76–91
	SF (batch)	*Pistia stratiotes*[a]	1 cell, 10 dm^3	15	–	1000–5000	-	88–96
Nelson et al. (2006)	SF	*Schoenoplectus californicus*	4 pairs of 4000 m^2 cells	2	–	30–40	2–8	75–82
Khan et al. (2009)	SF	Mix of 11 species	7 cells (4145 m^2)	1.7	–	1450	750	48
Kropfelova et al. (2009)	HSSF	*Phragmites australis/Phalaris arundinacea*	4 cells, 3520 m^2	–	108	25	6.6	74
	HSSF	*Phragmites australis/Phalaris arundinacea*	2 cells, 504 m^2	–	16	41	6.5	84
	HSSF	*Phragmites australis*	2 cells, 983 m^2	–	34	25	15	42
Megateli et al. (2009)	SF (batch)	*Lemna gibba*[a]	1 cell, 0.25 dm^3	10	–	0.1	0.02[b]	81
	SF (batch)	*Lemna gibba*[a]	1 cell, 0.25 dm^3	10	–	100	33[b]	77
Yeh et al. (2009)	SF (batch)	*Typha latifolia*	1 cell, 0.18 × 0.5 × 0.5	1.25	0.144	64	11	83
	SF (batch)	*Phragmites australis*	1 cell, 0.18 × 0.5 × 0.5	1.25	0.144	64	11	83
Wojciechowska and Waara (2011)	2 VSSF + 1 HSSF	*Phragmites australis*	Two VSSF (7.5 and 5.0 m^2) followed by a HSSF (12.25 m^2).	–	100	80	20	73
Sekomo et al. (2012)	SF (batch)	*Lemna minor*[a]	3 cells in line, each of 0.5 × 0.3 × 0.3	7	–	110	80	18
	SF (batch)	*Lemna minor*[a]	3 cells in line, each of 0.5 × 0.3 × 0.3	7	–	240	200	17
	SF (batch)	*Lemna minor*[a]	3 cells in line, each of 0.5 × 0.3 × 0.3	7	–	250	190	24
Anning et al. (2013)	HSSF	*Thalia geniculata*	2 cells in line, each of 2.1 × 1 × 0.8	5–7	1	310	279[b]	10
	HSSF	*Typha latifolia*	2 cells in line, each of 2.1 × 1 × 0.8	5–7	1	310	205[b]	34
	HSSF	*Limnocharis flava*	2 cells in line, each of 2.1 × 1 × 0.8	5–7	1	310	298[b]	4

W, winter; S, summer.
[a] Floating and/or submerged macrophytes.
[b] Our own calculation.

wetlands planted with *Arundo donax* and *Acorus calamus* than in unplanted wetlands. The DOM and microbial biofilms in unplanted CW may bind Cu^{2+} and trap Cu oxides. In planted CW, the DOM, microbial biofilms, macrophyte roots and root exudates can have similar effects. The higher Cu^{2+} removal rate of S#2 compared to S#1 may be due to higher plant and microbial activities at the beginning of the growing season than in late winter.

Total Cu but also Cu^{2+} removals were lower in CW planted with *J. articulatus* than in other treatments. In our CW, *J. articulatus* produced similar belowground biomass than *P. australis* and *P. arundinacea* (personal data) and thus may offer similar sorption sites onto roots. *J. articulatus* may maintain Cu in solution through Cu complexation with LMMOA (low molecular mass organic acid) such as oxalate released by *Juncus maritimus* with rising Cu exposure (Mucha et al., 2010) and consequently may decrease total Cu removal. In addition, *J. articulatus* can release allelopathic compounds (Dakora and Phillips, 2002; Ervin and Wetzel, 2003) which may constrain the biofilm development (Gross, 2003; Zhang et al., 2009).

Macrophytes used in this study were sampled at a Cu-contaminated site. As suggested by Brisson and Chazarenc (2009) and Marchand et al. (2010), CW designs for metal removal may consider the selection of macrophyte species but also ecotypes whose TE tolerance depends on the growing location (Marchand et al., 2010). However it is not the only criterion. In our study, *P. australis* suffered aphid attacks in both 2011 and 2012, leading to a massive death of plants in CW at the end of growing season (weeks 35–40) whereas *P. arundinacea* and *J. articulatus* were not affected by aphids. Such *P. australis* low resistance to aphids at the end of growing season in temperate climates could be included in plant selection strategies.

3.2. Physico-chemical parameters and Cu removal in bio-racks during a 14-day period in acidic conditions

In S#3, pH was set to 6.1 at day 0. It progressively increased – similarly in all treatments – from 6.8 ± 0.2 (*J. articulatus*) to 7.4 ± 0.3 (control) at day 3, reached 7.8 ± 0.3 at day 6, and was in the [7.8 ± 0.1 to 8.1 ± 0.1] range for all treatments at day 14 (Table 1). Simultaneously, redox potential did not significantly vary, staying in the 210.3 ± 5.8 mV (*P. australis* at day 10) – 238.2 ± 2.4 mV (*P. australis* at day 3) range (Table 1). Cu in water would be thus mainly in Cu^{2+} form for S#3. Electrical conductivity increased in all CW, from 554.8 ± 16.7 to 850.2 ± 96.5 $\mu S\,cm^{-1}$ in CW respectively planted with *P. arundinacea* at day 0 and unplanted at day 14. Unplanted CW presented the highest EC values during the whole S#3 experiment. This likely resulted in metal desorption under acidic conditions. Total Cu concentrations in water increased in all treatments, from 1.6 μM (103.5 ± 26.8 $\mu g\,dm^{-3}$) to 2.85 μM (181.4 ± 48 $\mu g\,dm^{-3}$) respectively in CW planted with *P. australis* and *J. articulatus* at day 0. Free Cu^{2+} concentrations in CW water also increased compared with S#1 and S#2 to reach 1.4 μM (90.1 ± 48 $\mu g\,dm^{-3}$) in CW planted with *J. articulatus*, *P. arundinacea* and *P. australis* while this concentration was 0.9 μM (57.5 ± 8 $\mu g\ dm^{-3}$) in the unplanted control at day 0. At S#3 start, free Cu^{2+} represented 73% and 86% of the total Cu in CW planted with *P. australis* and *P. arundinacea*, 49% in CW planted with *J. articulatus* and 43% in unplanted CW (Table 2). Thus, Cu desorption from roots and root exudates may be higher than Cu desorption from the microbial biofilm. Increase in pH for all CW during the S#3 may result from H^+ competition with Cu previously sorbed on the various CW components, i.e. perlite and Fe oxides of diorite, DOM, roots, microbial biofilms, and CW walls. In parallel, roots may excrete OH^- and HCO_3^- to buffer water pH and to take up anions such as nitrates and sulfates (Dakora and Phillips, 2002). However, water pH was similar in all CW (Table 2) and thus roots may less influence it compared to other CW components.

After 6 days, once excess H^+ was sorbed on the perlite, Fe oxides, DOM, microbial biofilms, CW walls and roots in planted CW, the overall mean surface charge of ferric (oxyhydr)oxides changed from a positive to a negative value as pH started to increase (Sheoran and Sheoran, 2006). Thus Cu^{2+} may adsorb again on diorite Fe oxides and silicates provided by the perlite. Copper may also adsorb again onto the root plaque, PVC walls and the microbial biofilm. Similarly, Ferris et al. (1989) reported that biofilm metal uptake at neutral pH was enhanced by up to 12 magnitude orders over acidic conditions and that adsorption strength values were usually higher at elevated pH. At day 14 in S#3, total Cu concentration in water decreased to 0.5 (31.4 ± 7), 0.3 (18.5 ± 1.8), 0.2 (13.2 ± 7), and 0.2 (13.5 ± 4) μM (and $\mu g\,dm^{-3}$ under brackets) respectively in CW planted with *J. articulatus*, *P. australis*, *P. arundinacea* and the unplanted CW (Fig. 2 and Table 2). Free Cu^{2+} concentration in water decreased to 0.025 (1.6 ± 0.5), 0.012 (0.80 ± 0.5), 0.010 (0.65 ± 0.2), and 0.015 (0.98 ± 0.5) μM ($\mu g\,dm^{-3}$) respectively in CW planted with *J. articulatus*, *P. arundinacea*, *P. australis* and unplanted CW (Fig. 3 and Table 2). In all CW, the removal rate of Cu^{2+} reached 99%, and consequently the RTEI was 0 for the three macrophytes. Such RTEI values would indicate in overall no influence of rooted macrophytes on the Cu removal rate in acidic conditions for such "young" CW, although some mechanisms may offset each other.

Various designs used for the construction of constructed wetlands are a major source of variability of physico-chemical parameters in CW. Therefore, it is difficult to clearly compare the effectiveness of two CW in terms of elimination of TE such as Cu. However, Cu removal rates in our three experiments are in line with previous findings (Table 3). For CW planted with *P. australis*, the total Cu removal rate was respectively 43% in winter and 56% in summer (Samecka-Cymerman et al., 2004), 42% (Kropfelova et al., 2009), 79% (Mantovi et al., 2003) and up to 83% (Yeh et al., 2009). In two HSSF planted with both *P. australis* and *P. arundinacea*, total Cu removal rates were 74 and 84% respectively (Kropfelova et al., 2009). The higher total Cu and Cu^{2+} removal rates of S#3 compared to S#1 and S#2 in our study may be due to higher plant and microbial density and activity just after the peak of the growing season (Faulwetter et al., 2009; Chazarenc et al., 2010). Thus, even in "young" CWs, sorption sites provided by the substrate and those related to the bacterial biofilm are both key players involved in Cu removal in CWs. This confirm previous findings reporting the distribution rates of total Cu load into the effluent (75%), algae (1%) and the final resident biofilm (19%) in algal ponds free of sediment (Sekomo et al., 2012).

4. Conclusions

Pilot constructed wetlands (CWs) using bio-racks for cleaning synthetic Cu-contaminated wastewaters (2.5 μM Cu) were carried out in alkaline (S#1 and S#2 experiments) and acidic (S#3) conditions for assessing the influences of three macrophytes on the removal rate of Cu. All macrophytes well developed in the bio-rack system and provided a high root surface for the growth of microbial populations in oxidative conditions. Various compartments, i.e. diorite, Fe oxides, perlite, microbial biofilms, PVC walls, roots and rhizomes are likely key-players for water Cu-decontamination in such CWs. The influence of the three macrophytes on the Cu removal rate was low, and even negative for *J. articulatus*, compared to the unplanted CW. *J. articulatus* may maintain Cu in solution through Cu complexation with LMMOA and release of allelopathic compounds which may constrain the biofilm development. However, macrophyte supply of organic matter (OM) involved in TE

removal by plants in CW is often overlooked in short term studies and is generally higher at mid- and long-term. Further investigations should determine at what time macrophytes have an influence as a major OM source in CW. The use of the Relative Treatment Efficiency Index (RTEI) to assess plant importance in CW is recommended. Choice of macrophytes may also depend on the resistance of macrophytes to pathogens attacks. Here, *P. arundinacea* better resisted to aphids than *P. australis*. Constructed wetlands also need maintenance to avoid clogging over time due to OM accumulation, biofilm development and probably saturation of sorption site. Bio-racks offer a new way to rejuvenate the CW by periodically replacing some of the columns. The French Water Agency (FWA) has defined an upper critical threshold value (<10 μg Cu dm^{-3}) for a good freshwater quality (SEQ EAU, 2003). Recirculation of Cu-contaminated water (2.5 μM, 158.5 μg Cu dm^{-3}) in our bio-rack based-CW allowed reaching 0.8 μM (48 μg Cu dm^{-3}) in early spring and 0.2 μM (13 μg Cu dm^{-3}) as the growing season peaked. The FWA standards may be reached by extending the residence time during the growing season, but in winter low microbial and root activities are of concern. The selection of rhizosphere bacteria tolerant to cold conditions is an option (Gilbert et al., 2012). Further investigations are needed to characterize sorption and biological reactions as well as microbial biofilms and their interactions with Cu in our CW at short-, mid- and long term.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecoleng.2013.12.017.

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